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Nanotechnology: A New Hope for the Cure of Osteoarthritis, Osteoporosis and Rheumatoid Arthritis

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Abstract *Bone and cartilage diseases especially osteoporosis, osteoarthritis and rheumatoid arthritis are rapidly prevailing both in men and women particularly due to increase in life expectancies. Different treatments are being proposed using conventional drugs and their modifications. But the side effects associated with such drugs and difficulty in treatment strategies due to multifactorial nature of such diseases and difficulty in drug delivery led the researchers towards the development of more advanced technologies for the treatment purpose. Nanotechnology is a promising strategy for treating such diseases that suppresses the progression of such diseases providing causal treatment. In this review, we will summarize the recent nano-based targeted and non-targeted delivery systems using various types of nanoparticles, nanogels, nanocomplexes, nanocarriers, hydrogels etc. for the efficient delivery of drugs and other therapeutic agents like mRNA, genes, insulin-growth factors etc. Moreover, role of nanoparticles for bone and cartilage repair in tissue engineering will also be discussed.*

Key Words: Bone Repair, Nanoparticle, Nanotechnology, Osteoarthritis, Osteoporosis, Rheumatoid Arthritis, Tissue Engineering

Introduction

Skeletal associated diseases have always been an important topic among the researchers due to its frequent occurrence in both men and women. Such diseases are rapidly spreading due to increase in life expectancies. Among them, osteoporosis, osteoarthritis, and rheumatoid arthritis are considered most prevailing especially in adult women (Rabiei et. al., 2020). Rheumatoid arthritis is a chronic autoimmune disorder marked by pain and inflammation in joints (Prasad, O'Mary & Cui, 2020). Osteoporosis on the other hand, is a musculoskeletal disease in which either the deficiency in vitamin D or calcium and the hormonal changes make the bone tissues fragile to the extent that the endpoint is bone fracture (Mackey & Whitaker, 2015). Osteoarthritis is characterized by joint degradation and painful osteophyte development in the surrounding tissues (Mohammadinejad et. al., 2020). Conventional drugs have already been used for treating such diseases but due to the multifactorial nature of such diseases involving complex biochemical pathways, the need for the extensive study was made inevitable. The insufficient availability of drug to the target site, rapid removal from the synovial cavity, off-target side effects and many more limitations associated with conventional delivery of drugs led us to more advanced treatments of such diseases (Chowdhury et. al., 2017). Moreover, many such drugs provide palliative relief and they offer temporary treatments (Prasad, O'Mary & Cui, 2015).

Nanotechnology has now become a center of attraction for its wide applications in medicine,

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especially in the advancement of drug delivery systems. Nanocarriers play an important role to overcome the limitations of conventional drug delivery by improving the stability, reducing side effects, and releasing drugs in a sustained manner (Gouveia et. al., 2015). In this way, the targeted-drug delivery can also be achieved. With the advancement of this technology, various nanoparticles are used for drug delivery like chitosan, polymeric, gold, albumin-bound, antibody conjugated and many more are also used (Bottini et. al., 2016; Cho et al., 2014). Now, it has become possible to replace the defective tissues by using the scaffolds that are manufactured from nanotubes, nanoparticles, nanofibers, and hydrogels (Lu et. al., 2018). In this review article the role of nanotechnology will be discussed to enhance the drug delivery in osteoarthritis, osteoporosis, and rheumatoid arthritis- both targeted and non-targeted. This article provides the importance of tissue engineering for cartilage and bone repair. Moreover, how this recent pharmaceutical technology overcame the limitations of conventional drugs will also be discussed.

Limitations Associated with Conventional based Drug Delivery to Skeletal System

Due to the complex pathways and multiple factors involved in the skeletal diseases, conventional drugs fail in many aspects (Fang et. al., 2019). In the osteoarthritis of bone, there is insufficient delivery of drugs to target sites (Lawson et. al., 2020). The drug concentration at the target site is reduced and it is increased near the superficial layers of cartilage when intra-articular injection is administered in knee joint, thereby leading to systemic toxicity. Drug administered locally at joints shows rapid clearance from the synovial cavity. Moreover, the off-target drug accumulation leading to clearance of drug from the lymphatic vessel, electrical and physical barriers in extracellular matrix and avascularity leading to the reduction of bioavailability of drugs in cartilage also impose serious complications (Feng, & Chen, 2018). The size of the drug also affects the clearance of drug. Frequent injection of drugs into the arthritic joints serve as a disadvantage of providing worse patient compliance or discomfort and also increase the risks of infection at the injection site. When bone cement is injected, it induces some issues related to biocompatibility.

Large amount of filtration is experienced by intra-articular fluid through the capillaries and then enter the joint cavity and also undergoes drainage into lymphatic space from synovial cavity. This limits the intra-articular injection due to the reduction of the lifespan of the drug in the cartilage (Nasiri et. al., 2019). The hydrophobic drugs are more difficult to deliver due to the aqueous nature of the synovial fluid. On the contrary, pressure gradient causes an increased clearance of hydrophilic drugs from the joints. The most prominent limitation of rheumatoid arthritis treatment is that it suppresses the immune system leading to drug-associated side effects. (Ain et. al.,2019). These limitations have been overcome by Nanotechnology involved in the treatment.

Improved Drug Delivery to Skeletal System by using Nanotechnology

The use of nanomaterials has overcome the challenges of drug delivery to cartilages and bones because of their smaller size, low chances of phagocytosis and high retention capacity. This can be achieved by targeting nanoparticles by their attachment to specific cells such as the inflammatory agents and chondrocytes (Dolati et. al., 2016). These nanoparticles carry positive charge attaching itself to negatively charged cartilage and in this way, it avoids filtration from the cartilage pores.

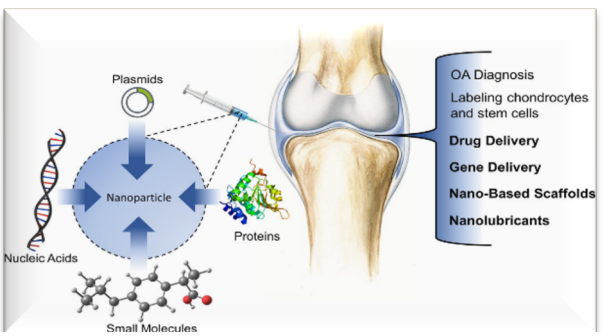

Figure 1: Nanotechnology have multiple applications in osteoarthritis starting from diagnosis to the delivery of drug to targeted sites (Lawson et. al., 2020).

osteoarthritis, passive delivery becomes useless due to angiogenesis in which limited fenestration of blood capillaries are present. (Eichaker et. al., 2014). Thus, active target delivery is preferred. Nanocarrier that targets the specific site is more suitable (Brown, Kumar & Sharma, 2019). It becomes available in the extracellular matrix of cartilage, where it delivers higher doses of inflammatory pathways inhibitors and also reduces the drug clearance by macrophages. The wide range of role of NPs in osteoarthritis has been shown in Fig 1.

In rheumatoid arthritis, fenestrated capillaries are present, lymphatic leakage occurs and it leads to enhanced retention effect and enhanced permeability. The abundant fenestrations are present due to the presence of defective angiogenesis in rheumatoid arthritis, which in return eases the passive drug delivery. Nanoparticles can be coated with hydrophilic polymers which prevent the fast clearance from kidneys and it also prevents degradation of nanodrug and in this way it facilitates the accumulation of drug in the inflammatory joints (Burmester & Pope, 2017). In rheumatoid treatment, the target drug delivery is achieved in such a way that they recognize markers on macrophages and lymphocytes and attracts and inhibits the macrophages and lymphocyte proliferation respectively (Zhang et. al., 2018). siRNA and different drugs are used for the inhibition of cytokines ultimately activate the cartilage degeneration. These drugs are transported by NPs Fig 2.

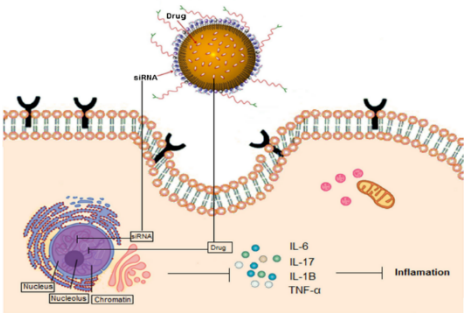

Figure 2: Approach of Nanoparticles (NPs) in Rheumatoid Arthritis. Various nanoparticles are used for its treatment. siRNA and the drugs that work against cytokines are transported by these nanoparticles preventing the pro-inflammatory cytokines expression and inhibiting the degeneration of cartilage and inflammatory activation (Dolati et. al., 2016).

Osteoporosis for many decades was believed to be caused by estrogen loss especially in old women. This finding lead to the more advanced techniques that are hormone based for the purpose of regeneration and scaffold-based treatments were also considered important for the osteoclast activity inhibition and differentiation of osteoblast. It has been recently studied that when beta-estradiol is loaded into mesoporous silica nanoparticles, it enhances the nanomaterial osteoconductivity when it is implanted on the titanium substrate (Barry et. al., 2016). Moreover, the role of NPs, bioengineered materials for the bone regeneration and bone remodeling purpose along with combinatorial therapy has been shown in Fig 3 & Table 1.

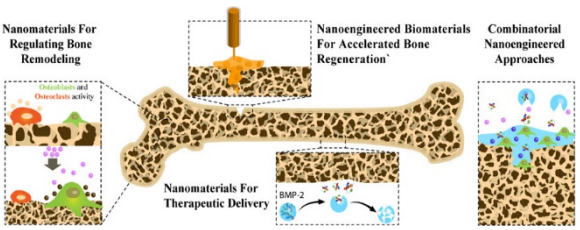

Figure 3: Nanotechnology is used in the treatment of osteoporosis. (a) therapeutic delivery through nanoparticles (b) Regulation of bone remodeling through nanoparticles (c) for acceleration of bone regeneration, usage of nanoengineered biomaterials (d) Combinatorial nano-engineering for OSP treatment (Barry et. al., 2016).

Table 1. Recent Nano-based treatment strategies for Osteoporosis

Treatment Method	Advantages
Nanomaterials (for therapeutic delivery) (Wei et. al., 2016)	Aid in new bone formation
Nanomaterials (for bone remodeling regulation) (Barry et. al., 2016)	For bone resorption inhibition and decreased osteoclast activity, materials like bisphosphonates were designed. Studies of different nanomaterials such as synthetic silicates & calcium phosphate-based materials for stem cell differentiation were carried out.
Nanoengineered biomaterials (for bone regeneration) (Barry et. al., 2016)	Nano-based biomaterials such as nano-hydroxyapatite & silicate nanoparticles (siNPs) can be used to implant in a biocompatible scaffold and hence promote mechanical strength of the matrix and differentiation of osteoclast.
Combinatorial nanoengineering (Barry et. al., 2016)	When osteoconductive ability of nanomaterials such as siNPs, nHA, LDHs is harnessed, it promotes new bone formation when it is implanted in a hydrogel scaffold.

Targeted and the Non-Targeted drug Delivery

The limitations and short comings in the conventional drugs have led to the development of advanced drug delivery techniques in the concerned fields. (Gu, Wu, Chen, 2013). Accumulation of drug in liver and spleen is the major drawback of non-targeted drug delivery. In such cases, non-targeted drugs e.g. curcumin have become important as they do not impose serious side effects (Samarasinghe, Kanwar & Kanwar, 2014).

The targeted action of methotrexate and prednisolone impose severe side effects in the arthritic tissues (Trujillo-Nolasco et. al., 2019). Several studies have been carried out such as dextran sulphate-grafted- MTX which was prepared to specially target the scavenger receptor (SR) present on the surface of macrophages that are overexpressed in order to treat Rheumatoid arthritis (Roy, Kanwar & Kanwar, 2015). This DS-g-MTX forms micelles having the diameters of about 100nm and is used in collagen-induced arthritis for effective active type of targeting under ex-vivo and in-vivo conditions (Yang et. al., 2017). As the cartilage is of avascular nature, the delivery of drugs via circulation at such site is almost ineffective. This nature can be used for the fast efflux of the drugs from the synovial joint and in this way the required outcome of the drugs cannot be achieved. So, the arthritis can be treated in such a way that we target drugs to the extracellular membrane present in the tissues and it has proven to be vital and most efficient way of treating arthritis due to the large half-lives of the molecules of ECM in the joints (Schultz, 2019). This way of targeting drugs provides broad therapeutic window and hence drug retention can be enhanced and prolonged. The components of ECM of cartilage show wide attraction with the peptides; for example, heparin-binding peptides and aggrecan binding peptides and hence improve the drug retention (Li et. al., 2018). Presently, small molecule disease-modifying anti-rheumatic drugs are used as a first line of treatment, but their side-effects of long-term administration restrict their use in clinical practice. Nanomedicines are used to deliver drugs to the targeted cells and tissues either actively or passively. Ideal properties of nanomedicine in active targeting for the treatment of RA included surface charge (anionic/neutral), suitable size, PEGylation to increase circulation time and modification using appropriate ligands.

ROS, found in the synovium, can be used as a target in targeted delivery in arthritis and enhance the conversion of hydrophilic to hydrophobic drugs in polypropylene sulfide. These oxidizing species also stimulate curcumin from capsules of polypropylene sulfide to show anti-inflammatory effect (Fan et. al., 2020). Curcumin can be very effective when it is provided in the form of nano-based formulations (Kang et. al., 2020; Yallapu et. al., 2015). Hence these proteinases can be targeted for the treatment of the concerned diseases. The anti-arthritic drugs can be topically applied and overcomes the degradation limitation by the degradative pathways, reduced toxic effects, good patient compliance, controlled and sustained drug delivery. The common conventional medicines that are employed in the treatment of osteoarthritis and rheumatoid arthritis are NSAIDs such as aceclofenac, diflunisal, diclofenac, meloxicam (Xiao & Chen, 2020). As NSAIDs show gastrointestinal associated side effects, their preferred route of drug delivery is transdermal because in this way the drugs

are delivered at the site of action. The use of nanoparticles in NSAIDs, the sustained release of these drugs can be enhanced (Garg et. al., 2017). But the main barrier present in the transdermal drug delivery is the stratum corneum barrier which can be overcome by vesicular drug delivery (Kaur et. al., 2017). Nanoformulations have proven to be excellent way of drug delivery to penetrate in the skin layers that are too deep for the conventional drugs not to be reached (Wang, 2019). The examples of nanoformulations include floxosomes, tranferosome and solid-lipid nanoparticles. For targeted and non-targeted drug delivery to the arthritic site's different nanoparticles and nanocarriers are used. Table 1, 2.

Chitosan-Based Nanoparticles

They are used for targeted drug and gene delivery. They are biodegradable and biocompatible polymers which act as nanocarriers for polymeric nanoparticles for the targeted drug delivery (Mohammed et. al., 2017). They are naturally occurring polymers mainly of polysaccharides. It is attractively studied for the encapsulation of quantum dots due to its properties of chelation of metal ions and water solubility. It is well known for its targeted gene and drug delivery by its conjugation to transferrin 1, folic acid, siRNA/NPs inhibited by notch 1can cause decreased inflammation proliferation, cartilage damage and bone erosion (Kim, 2015). KGN-coated chitosan nanoparticles can be used to enhance cartilage regeneration.

Parathyroid hormone when incorporated in the chitosan-based nanoparticles causes the stimulation of osteogenesis and it also contributes in the reduction of gastrointestinal associated adverse effects. It is because these nanoparticles cause the absorption of the Hydrogen ions by amine groups that are on the chitosan in stomach. Coating of chitosan and parathyroid hormone complex with polyethylene glycol enhances the surface characteristics and stability of nanoparticles in stomach and also improves bioavailability and facilitation of drug transport (Mohammed et al., 2017).

Liposomes

They are considered as spherical vesicles having single or multiple layers of lipids responsible for assembling them in aqueous systems. NSAIDs can be nano-encapsulated to provide enhanced anti-inflammatory effects by decreasing the serum concentration of inflammatory cytokines like IL-6 and TNF-alpha. Polyethylene glycol liposomes lead to the complete treatment of inflammation by a single systemic administration (Kapoor et. al., 2014). siRNA and cationic liposomes complex can be used to fight against certain cytokines like IL-1, IL-8, IL-8, and TNF-alpha. (Monteiro et. at., 2014).

Polymeric Nanoparticles

They are made from biocompatible and biodegradable polymers. Such polymers can encapsulate the hydrophobic and hydrophilic molecules of drug. The advantage of such nanoparticles is its sustained release and less frequent administration (Dubey et. al., 2017). For encapsulation of beta-methasone, combination of nanoparticles of poly (D,L-lactic /glycolic (PLGA) / poly (D,L-lactic acid); (PLA) form homopolymers and copolymers are formed by PEG-PLGA/PLA leading to an enhanced effect of glucocorticoids in the treatment of arthritis. When hyaluronic acid is combined with PLGA particles, they serve as a treatment of osteoarthritis causing viscosupplementation (Mota et. al., 2019).

Gold Nanoparticles (AU-NPS)

Gold nanoparticles serve as an emerging paradigm for the purpose of targeted bases and non-targeted delivery of drugs (Kumar, Zhang & Liang, 2013). Gold nanoparticles such as gold salts possess anti-angiogenic properties when it is conjugated with other biologics or DMARDs. It was also investigated in the reduction of side effects of inflammation (Tarner & Muller, 2008). When gold nanoparticles are conjugated with cyclo-dextrin (CD) that contains Curcumin, results in the formation of a complex which precludes the formation of osteoclast from the bone marrow derived macrophages. RANKL activator is inhibited by drug delivery of this type. RANKL receptors are found on the surfaces of pre-osteoclasts and also the mature osteoclasts. The bone resorption enhancement by the osteoclasts in the rheumatoid arthritis condition occur where such RANKL expression can be found. Moreover, when Hyaluronate gold nanoparticles are conjugated to form the complex with tocilizumab (an antibiotic), then this complex can be used to treat rheumatoid arthritis efficiently (Lee et. al., 2014).

Albumin-bound Nanoparticles

The ability of albumin-bound nanoparticles to accumulate in inflamed tissues make them beneficial to be used in arthritic patients for arthritis targeting. A significant decrease in cartilage degradation and synovial fibroblast invasion results by using

methotrexate and human serum albumin conjugate. (Fiehn et. al., 2004). TAC solution used for anti-arthritic activity when administered alone does not exhibit its activity as much as TAC-loaded human serum albumin (HSA) nanoparticles (Syed & Devi, 2019). Hyaluronic acid that is pre-coated with bovine serum albumin nanoparticles that are also loaded with brucine can act as nano-vectors for the target drug delivery in the case of intra-articular injection (Chen et. al., 2013).

Antibody conjugated Nanoparticles

It is targeted type of drug delivery. The major advantage of such nanoparticles is to enhance the cell penetration of the antibodies and to bind to their target sites with high affinity. The ligand-targeted therapy is achieved due to the molecule's association such as antibodies to nanosystem. Such ligands can bind to target cells whose receptors can be excessively expressed or they are unique as compared to normal tissues (M Cardoso, N Peca & CA Rogue, 2012).

Magnetic Nanoparticles

Magnetic nanoparticles due to their extreme small size show the efficient cellular uptake and the drug bio-distribution and in turn enhance the efficiency of drug delivery (Zhang et. al., 2018). Magnetic nanoparticles possess some advantageous characteristics like they have unique magnetic properties, unique electrical properties and possess specific dimensions to enhance the regeneration of the bone and cartilage (Gao et. al., 2015). Magnetic iron oxide nanoparticles have proven to be structurally stable in the mesenchymal stem cells (MSCs) and have shown excellent results regarding the differentiation of mesenchymal stem cells and this is known as osteogenic differentiation (Wang et. al., 2017).

Avidin Nanocarriers

They are the excellent nanocarriers used for intra-articular drug delivery. It is because of their extreme small size and bearing a positive charge on them that eases the quick penetration of drug through the thick cartilage and they allow the drugs to bind electrostatically to their target sites to provide long half-lives (Bajpayee et. al., 2016). Avidin nanocarrier is also used for the intra-articular delivery of the drug dexamethasone (DEX) through which DEX is rapidly released from DEX-nanocarrier conjugates and show high bioactivity ameliorating the catabolic associated effects of cytokine in osteoarthritis. (Bajpayee et. al., 2016)

Nanoplexes

Nanoplexes can be used for the target delivery of insulin-like growth factor-1. Nanoplexes are the types of complexes that incorporate poly-anionic layers of glutamic acid and poly-cationic layers of arginine and making it easy for the drugs to deeply penetrate the cartilage so that extracellular membrane can be targeted.

Nanocomposite Hydrogels

Nanocomposite hydrogels, which utilizes the positive aspects of both hydrogel matrices as well as nanofillers have showed better biological and mechanical properties and is expected to bring revolutionary advancement in biomedical field in near future. For better performance of NC gels, biological properties like cell differentiation, cell adhesion and protein adsorption should also be taken into consideration along with physical and chemical properties during the designing of NC. (Song et. al., 2015).

Biopolymer Dependent Nanoparticles

Biopolymers are preferred over synthetic polymers due to a number of characteristics like biodegradability, biocompatibility, and excessive renewable sources. As size and distribution of particles are most important factors for targeted drug delivery, proteins are most preferred to be used as drug or gene carriers because of their definite molecular size and ability to form self-assembled and random structures which allows them to form distinctive nanostructures. Polysaccharides are also favorable candidates for production of nanoparticles. Adding ligands on the surface of nanoparticles for targeted drug delivery enhances the efficiency of biopolymer nanoparticles and increases its use in clinical practices (Nitta & Numata, 2013).

Lipid-Based Nanoparticles

The major advantage of lipid-based nanoparticles is its least toxicity among other nanoparticles for the in-vivo applications (Puri et. Al., 2009). Both DNA/RNA based, and drug-based delivery systems have been proved to be used in such nanoparticles to enhance their ability. Lipid-based nanoparticles provide biocompatible drug delivery systems by targeting over the free drugs when

it presents the targeting of tissue-specific ligand-controlled release of drug (Chuang et. Al., 2018).

Graphene-Based Nanoparticles

Graphene based nanoparticles have shown unique characteristics in tissue engineering and the delivery of drugs (Goenka, Sant & Sant, 2014). Graphene is a single sheet of two-dimensional layer that show excellent mechanical and electrical properties. The nano-hydroxyapatite/graphene nanoribbon complex serves as an excellent scaffold material for regeneration of bone because of its favorable properties like good bioactivity and osteo-integration properties (Oliveira et. al., 2019). Graphene oxide-based nanoparticles not only shows its activity in drug delivery but also offers cartilage protection when it interferes with Rank/Rank1/OPG pathway (Cheng et. al., 2018).

Nanocrystal Polymer Particles

They act as drug delivery carriers for the treatment. Nanocrystals of keratogenin (KGN) that are loaded with polymer microparticles showed prolonged drug release and sustained persistence in intra-articular space without irritation (Maudens et. al., 2018). KGN-conjugated polyurethane nanoparticles (NP-KGN) are used for the treatment of osteoarthritis by releasing KGN in a sustained manner. They decrease cartilage degeneration efficiently.

Polypeptide Nanogels

Polypeptide nanogels (PNGs) with encapsulation of methotrexate has proven to be useful for the improvement of collagen-induced arthritis. This conjugation shows enhanced glutathione (GSH)-triggered behavior, high toxicity against the activated macrophages, decreased inflammatory cells, widening of joint space and decrease in the articular surface roughening. In rheumatoid arthritis, the hypoxic condition is generated that affects the joints and consequently increase the glutathione (GSH) in the arthritic joint (Zha, Banik & Alexis, 2011). This GSH has the anti-oxidative stress activity which in turn, increases the release of therapeutic agents in rheumatoid arthritis. The reduction of GSH-triggered release behavior and responsiveness of the arthritic tissues is enhanced by using methotrexate loaded in methoxy polyethylene glycol-poly (L-phenylalanine-co-L-cystine) Mpeg-p (LP-co-LC) nanogel. (Pinelli et. al., 2020).

Table 2. Recent Nanotherapy approaches for drug delivery in Rheumatoid Arthritis (Chuang et. al., 2018)

Therapeutic Classification	Therapeutic Agents	Deliver/Target	Nanocarrier Systems
NSAIDs	Indomethacin	EPR	Lipid microspheres
	Aceclofenac	EPR	Lysine-based liposomes
	Indomethacin	EPR	Polymeric-based micelles
Biological agents	Tocilizumab	IL-6R+ cells	HA-gold NPs
	Anakinra	Macrophages	Folate-based chitosan NPs
Glucocorticoids	Dexamethasone	EPR	Liposomes
	Methylprednisolone	EPR	Cyclodextrin polymer
DMARDs	Methotrexate	EPR	Polymeric NPs that are stealth type
	Clodronate	Macrophages	Liposomes
Other inhibitors	γ —secretase inhibitor	Macrophages	Hyaluronan NPs
	Fumagillin	Integrin-activated cells	Carbon-based NPs

Table 3. Drug Delivery systems for the treatment of Osteoarthritis (Mohammadinejad et. al., 2020)

Nanocarriers	Therapeutic agents	Outcomes
PLGA NPs	WYRGRL peptide	Specifically bind to cartilage tissues & is biodegradable
Niosome	Date seed oil	Excellent anti-inflammatory activity, good stability
Bisphosphate NPs	Clondronate	Upregulates the gene expression of SOX9, reduced articular pain

Nanocarriers	Therapeutic agents	Outcomes
Lipid NPs	Ibuprofen	Greater entrapment efficiency, anti-inflammatory effect
Chitosan NPs	Berberine	Releasing profile is ideal & synovial fluid associated retention time
Solid lipid NPs	Aceclofenac	Offers prolonged release of drug and high drug uptake
Polymeric NPs	Curcumin	Inhibits the inflammatory mediators (TNF-$\alpha, IL-1\beta$) mRNA expression
Poly (ester-amide) particle	Celecoxib	No toxic effect at the site of injection, great biocompatibility
PEGylated NPs	KAFAK	Offers efficient drug target delivery
Nanocrystal polymer NPs	MAPK inhibitor	Target delivery, excellent retention time, decreased joint destruction

Nanoparticles Associated Drug, gene and Growth Factors Delivery for bone and Cartilage Regeneration

Nanoparticles have now recently been used to deliver a number of therapeutic agents to the components of skeletal system whether it is targeted or non-targeted. The examples of such therapeutic agents include parathyroid hormone and catabolic-cartilage factor, mRNA and genes and various types of growth factors. (Primardvand Chegini, Varshosaz & Taymouri, 2018). As discussed before, nanoparticles are used for the slow release of the drug in a continuous manner.

In osteoporosis, the targeted delivery of the nano-based drugs prevents the drug absorption in other organs. It also slows down or inhibits the progress of osteoporosis by enhancing the availability of calcium. Nano-hydroxyapatite (nHA) can also be used for treating osteoporosis. These nHA particles are periodically deposited in the collagen fibers which serves as a major role in the resistance of fracture by causing the superior mechanical heterogeneity (Qayoom, Teotia & Kumar, 2019). Poly (methyl methacrylate)-PMMA is considered as a bone cement. Its nanostructure is forcefully introduced by mechanical means in the bone to act as a filler and is one of the important treatments for osteoporosis. Zirconium dioxide and barium sulphate-based nanoparticles are used as additive agents to increase cohesion and biocompatibility of PMMA (Sabokbar et. al., 1997). The formation of HPG-IGF that is a human growth factor type 1 which is prostaglandin insulin like reduces and minimizes the two major limitations i.e. with the clearance of IGF-1 from the inflamed joints and that of systemic-based toxic effects (Loffredo et. al., 2014).

The use of nanoparticles in therapy that is of non-viral type reduces the limitations and disadvantages associated with the conventional viral based therapy e.g., immunogenicity, pathogenicity, carcinogenicity, toxicity (Jafari et. al., 2012). The most common non-viral based nanocarriers are the nucleic acid-lipid complexes (liposomes) and the complexes of gene with polymers known as polyplexes. Nanoparticles usage in the treatment of osteoarthritis instead of viruses has certain disadvantages like they are involved in the constriction of nucleic acids by the usage of cationic-based Polyethylene imine (PEI) as a gene-based nanocarrier (Evans & Huard, 2015). The use of PEI as non-viral gene carrier is beneficial as it provides high drug retention within the cartilage due to the interaction it possesses known as electrostatic interaction with hyaluronate which is negatively charged that forms crosslinks of hydrogel-like. Cationic type of polymers, for example poly-histidine and cationic-based Polyethylene imine are the excellent options for the regeneration of the components of skeletal system because of their ability to use transition metals for the gel formation (Lungwitz et. al., 2005).

Carbon nanotube (CTN) which is modified by using PEG (PEG-NT) and PEI is coated on it is used for the gene delivery to chondrocytes (Sacchetti et. al., 2014). PEG-NT is studied to break the extracellular barrier present on the cartilage and ultimately gets accumulated within the chondrocytes. (Suk et. al., 2016). Nanoparticles are also used in RNA-based drug delivery e.g., PEI that is linked to CAP which is the chondrocyte-affinity based peptide that causes the siRNA selective targeting to treat hypoxia inducible factor 2α (Hif-2α) found in chondrocytes (Pi et. al., 2015). The nanoparticle utilization to carry mRNA and siRNA is used for the protection of arthritic patients

from the nucleases and without showing any inflammatory-induced responses caused by RNA and also causes their absorption from the receptors found on cell surface (Sezlev et. al., 2019).

Natural polymers used in transporting the drugs to bone and cartilage also show an immense number of advantages like improvement in the targeting of drugs and genes to the target sites, repairment of cartilage, improvement in the effect of conventional injected drugs to achieve drug targeted delivery to cartilage. For example, chondroitin sulphate is a natural polymer having the therapeutic effect of proteoglycan synthesis, inhibition of the production of inflammatory cytokines and collagen II stimulation. This chondroitin sulphate has the ability to attach itself efficiently to the markers present on the surface of cell such as CD_{44}, P-selectin and β-selectin enhancing ability of attachment to cartilage cells and improving its delivery to the arthritic site via injection (Volpi, 2011). When Diacerein (DC) is used in conjugation with the chondroitin sulphate, the synergistic effect is produced which proves to be excellent therapy for osteoarthritis. This synergism happens in such a way that chondroitin sulphate causes an increase in the ability of DC to transform from its in-active form to active form that is known as rhein present in the cavity of synovium. The use of chondroitin sulphate for nanodrugs production causes enhanced chondrocyte regeneration and enhanced drug accumulation at the target site (Bishnoi et. al., 2016).

Polymeric-calcium phosphate cement (CPC) is used to treat bone defects but when silica nanoparticles are introduced in it, they enhance its strength and causes an increase in the proliferation of osteoblast. Poly (methyl methacrylate) (PMMA) matrix uses nanoparticles to increase its hardness and mechanical strength (Ricker, Liu-Snyder, Webster, 2008). Bone cement also incorporates the titanium dioxide (TiO_2) nanotube (TNT) for the improvement of mechanical properties of PMMA and causes the improved and more efficient antibiotics release (Shen et. al., 2019).

An experiment was done by the Osteoarthritis Research Society International (OARSI) in which they investigated that nanoparticles when used with Glycosaminoglycans (GAGs), showed the increase in the therapeutic activity of nanoparticles and consequently improved the osteoarthritis symptoms in the deeper parts of cartilage (Meneksedag-Erol, Tang & Uludag, 2018). Strontium also has the capacity to regenerate bones and prevents the bone resorption. The $SrTiO_3$ nanotubes results in two major advantages i.e. sustained delivery of strontium (Sr) and stimulation of osteogenesis (Zarins et. al., 2019).

Use of Nanomaterials for Cartilage and Bone Repair in Tissue Engineering

There are three prerequisites for the ex-vivo treatment of bone and cartilage disorders which include scaffold, growth factors and suitable stem cells (Jeon & Elisseeff, 2016). The scaffolds generally used in tissue engineering for cartilage and bone repair involve NPs having growth factors and stem cells. The NPs are stabilized by the growth factors which also protect them from decomposition. As a result, the NPs slowly deliver the growth factors and increase stem cells' proliferation as well as differentiation (Dubey et. Al., 2017). Ideally, the scaffolds used for reconstruction of articular cartilage should have appropriate mechanical strength and biodegradability and better biocompatibility (Walmsley et. al., 2015). The similarity in physical properties of injectable hydrogels and native ECM and slightly invasive procedures needed for their injection have made them in-demand scaffolds nowadays (Saiz et. al., 2013). Presently, in tissue engineering thermosensitive hydrogels are the center of interest because of their advantages like their ability to fill cartilage defects and prevent the undesired diffusion of precursor solution and gelling process can take place without the use of harsh environmental conditions and organic solvents unlike other injectable hydrogels (Scott et. al., 2006).

Furthermore, 3D scaffolds made up of polymeric matrix loaded drug can be injected as a fluid in the damaged cartilage and can be implanted surgically in the form of solid into the cartilage for cartilage formation. Permeability of cartilage explants can also be increased by NPs. Inside the cartilage explants, the neutral and charged particles stay effective for 1-15 days. NPs of specified size can enter the cartilage because the pore size is smaller than the distance between collagen fibrils present in cartilage ECM. Titania nanotubules (Ti-NTs) have proved to be preferable drug carriers because of their ability to release antibiotics effectively around the implants and increasing their biological activities. Antiosteoclastogenic and osteogenic properties can also be improved by Ti-NTs (Kim & Fisher, 2007).

Gene activated matrices can also be used for bone regeneration. In bone tissue engineering, GAM which is a neutral scaffold system having both viral as well as non-viral gene delivery vectors is widely used

to repair injuries (Webster & Ahn, 2006). In another procedure, recombinant adeno-associated virus (rAAV) has been used to transfer hTGF-β gene in order to treat OA. The expression of TGF-β will be increased in the cartilage by placing rAAV- hTGF-β within the micelle made up of polypropylene oxide and polyethylene oxide.

In tissue engineering, it has been proved that growth factors as well as Iron oxide NPs (IONPs) facilitate differentiation of Mesenchymal stem cells by affecting MSC genes. Moreover, IONPs are also able to repair tissues. Carbon compounds specially carbon nanomaterials e.g. graphene, fullerene and CNTs have the ability of direct differentiation of stem cells into bone cells as well as increase the life expectancy of MSCs in culture medium. Adhesion, proliferation, and migration of stem cells is controlled by carbon-based NPs (Abdal Dayem, Lee, & Cho, 2018). Cell differentiation is facilitated by the ability of these NPs to attract or repel differentiation factors and to enhance cell adhesion by the interaction among surface of carbon material and also the cell membrane (Webster & Ahn, 2006).

Conclusion

The nanotechnology has shown excellent applications in delivery of drugs to skeletal system which comprises of two categories. One is the delivery of drug to bone and cartilage and the other is the use of nanoparticles in tissue engineering. NPs are of various types like chitosan-based, gold-based, magnetic-based, graphene-based, polymeric, antibody-conjugated, liposomes and many more and are used for the targeted as well as non-targeted drug delivery. Nanogels, Nanoplexes, nanocarriers, hydrogels are also used for the efficient delivery of drugs to cartilage and bone. NPs aid in the efficient penetration along with accumulation of anti-arthritic drugs, growth factors, genes, and bone cement at the action site. Drugs are targeted to the extracellular membrane present in tissues which has proven to be the most efficient way of treating arthritis due to long half-lives of ECM molecules present in the joints. NPs have shown to overcome all the limitations of conventional drugs used for such diseases like it prevents rapid clearance of drugs from the synovial cavity and also prevents absorption by other organs promoting the retention of drugs at the site of action. Hence in this way required therapeutic efficacy is achieved with reduced side effects. These NPs are also used for the controlled release of drugs in the inflamed joints. As far as gene delivery is concerned, NPs are used for the prevention of toxicity, immunogenicity, carcinogenicity. They carry growth factors, mRNA, drugs, genes to the scaffold involved in tissue engineering. NPs have the ability to increase the permeability of cartilage explants and 3-D scaffolds implants. NPs that carry antibiotics or have the intrinsic antibacterial property cover the 3-D scaffold to provide protection against infection. Presently, thermosensitive hydrogels are also used in tissue engineering due to their ability to fill cartilage defects. Thus, nanotechnology has emerged as a promising treatment technology for osteoarthritis, osteoporosis, and rheumatoid arthritis.

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